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Chemical constituents, antioxidant, and antimicrobial activity of *Allium chinense* G. Don

Thejanuo Rhetso, R. Shubharani, M. S. Roopa and V. Sivaram*

Abstract

Background: *Allium chinense* G. Don is a medicinal and aromatic herb belonging to the family Amaryllidaceae, characterized by a high saponin content. The previous report has mostly been focused on the bulb, and there is very limited work on the leaf. The information about biological and chemical constituent of *A. chinense* leaf is still inadequate in contrast to the investigations reported on the bulb. To the best of our knowledge, there is no report on the hexane extract of both bulb and leaf extract. Therefore, the present investigation was focused on identifying and characterization of the hexane extracts of *A. chinense* bulb and leaf quantitatively and by using the GC-MS method and to know its scavenging, antibacterial, and antifungal activity.

Results: Twenty-eight bioactive compounds were identified in the bulb and nine in the leaf extract by GC-MS analysis. The versatile compounds present in the bulb are 2-methyloctacosane (21.30%), tetracontane (14.05%), eicosane, 10-methyl (12.06%), heneicosane (8.46%), octadecyl trifluoroacetate (6.48%), and 1-heneicosanol (5.76%), whereas in the leaf, it was phytol (35.76%), tetratetracontane (18.49%), perhydrofarnesyl acetone (14.76%), and heptadecane, 2,6-dimethyl (10.79%). In quantitative estimation, saponins were estimated to have the highest with 375.000 ± 0.577 mg/g in the leaf and 163.750 ± 0.433 mg/g in the bulb. The DPPH antioxidant scavenging activity was found to be minimum in both the bulb ($IC_{50} = 678.347$ μ g/ml) and leaf ($IC_{50} = 533.337$ μ g/ml). *A. chinense* extracts of both leaf and bulb exerted potential antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Leaf extract exhibited greater antifungal activity than the bulb against *Aspergillus niger*.

Conclusion: From the analysis, the hexane leaf extract exhibited higher antibacterial, antifungal, and antioxidant activity than the bulb. Their superior activity might be due to the higher content of total saponin and terpenes. This result will lead to further in-depth research towards the potential use of this plant; the bio-constituents can be further isolated and used in medical and therapeutic applications.

Keywords: Bulb extract, Leaf extract, GC-MS, Scavenging activity, Antibacterial, Antifungal

Background

Plants and their bioactive compounds are a potential source of medicine and are suitable with the prevailing demands for safe and effective treatment. Traditional knowledge procured over years of observation and interaction with the environment is a substantial source of modern medicine. Isolated and purified bioactive

constituents of the plant can be used for drug development in pharmacology and medicine.

The genus *Allium* belongs to the family Amaryllidaceae has 500 species and are characterized by their rich content of organo-sulfur as their main bioactive compounds [1]. *Allium chinense* (Fig. 1) is cultivated in the North-Eastern part of India region; the whole plant is edible raw or cooked and is used in culinary as a flavoring agent. The plant has a strong onion-like aroma and pungent taste, the entire plant is an expectorant,

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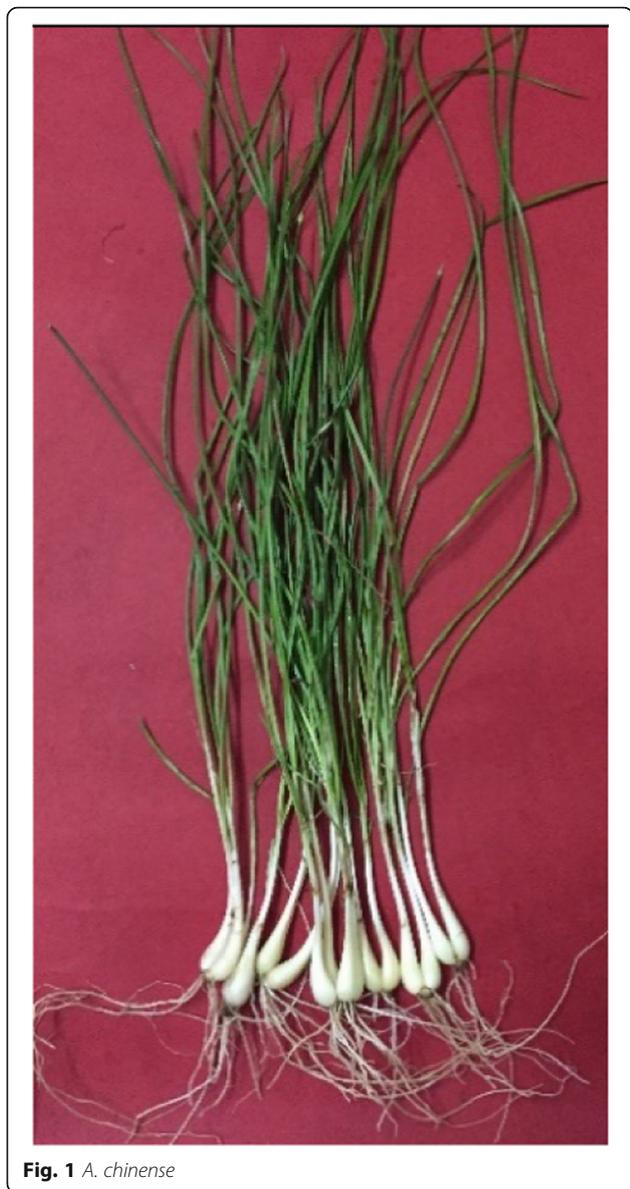


Fig. 1 *A. chinense*

carminative, and astringent also used to treat bronchitis, diarrhea, and angina pleurisy [2]. The bulb has high medicinal value in traditional Chinese medicine for over thousands of years and is the major source of the “Xie-bai” drug used in treating thoracic pain, stenocardia heart, asthma, and stagnant blood [3]. Among the many bioactive compounds present in *A. chinense*, the important active compounds are steroidal saponins, amino acids, sulfur, nitrogen, and flavonoids compound [4]. The earlier study on *A. chinense* leaf essential oil reported 34 organo-sulfur-containing compounds accounting 94% of the total volatiles [5]. The chemical investigation carried out in essential oil of the bulb reported a profuse content of sulfide compounds, as high as 97.71% [6]. Spirostanol saponins extracted from 60%

aqueous methanol extract [7] and laxogenin extracted from hot water extract [8] from *A. chinense* bulb have anti-tumor property. The protective effects of steroids from 60% ethanol bulb extract of *A. chinense* have great potential to prevent cardiac injuries induced by oxidative stress [9]. On literature survey, most of the earlier works have been carried out on the bulb and the biological and chemical report on *A. chinense* leaf is still scarce. However, no report to date has been found on hexane leaf and bulb extract of *A. chinense*. Therefore, the objective of the study was to characterize the chemical profile of the bulb and leaf hexane extract using GC-MS and the second objective was to determine the scavenging activity and antimicrobial activity of *A. chinense*.

Methods

Collection of plant material

Fresh and healthy plants of *A. chinense* were collected from the farmers in Kohima district, Nagaland, India. The collected plants were washed, carefully shade-dried till all the moisture contents were removed and ground to a fine powder using an electric grinder and stored in an airtight glass bottle at room temperature $27 \pm 2^\circ\text{C}$.

Extraction

A. chinense leaf and bulb were extracted separately using a soxhlet extractor with 200 ml hexane for 18–20 h. The extracts were separated from the solvent using a rotary evaporator at $30\text{--}40^\circ\text{C}$ and stored at 4°C for further use.

Quantitative analysis

Estimation of total alkaloid content

The total alkaloids were determined using the method described by Tan [10]. Atropine was used as a standard for plotting the calibration curve. The extracts (1 mg/ml) were dissolved in 2 N HCl and washed with chloroform, the layers were separated using a separating funnel, later bromocresol green solution and phosphate buffer (pH 4.7) were added and the mixture was vortexed, the yellow color complex formed at the bottom was pipetted out to measure the absorbance at 470 nm. The total alkaloid content was estimated using the linear regression equation obtained from the standard graph of atropine further mean \pm SD ($n = 3$) was calculated and expressed as mg/g atropine equivalent (AE).

Estimation of total flavonoids

Estimation of total flavonoids content was done using the aluminum trichloride (AlCl_3) method with different concentrations of quercetin as standard [11]. Extracts of 1 mg/ml were diluted with distilled water and 5% NaNO_2 solution was added. After being incubated for 6 min, 0.15 ml of AlCl_3 10% solution was added followed

by 2 ml of 10% NaOH, the final volume was adjusted to 5 ml using distilled water, and it was then incubated at room temperature for 15 min. Absorbance was read at 510 nm. All analysis was carried out in triplicate times and the total flavonoid content was calculated using linear regression equation obtained from the standard plot of quercetin and mean \pm SD ($n = 3$) was calculated. The result was expressed as mg/g quercetin equivalent (QE).

Estimation of total phenolic content

The total phenolics were estimated with Folin-Ciocalteu's method [12]. Gallic acid at varying concentrations was used as standard; plant extracts (1 mg/ml), 5 ml of FC (1:10) diluted reagent, and 4 ml sodium bicarbonate (7.5%) were taken in a test tube and the mixture was shaken and incubated in the dark for 30 min at 20 °C. The absorbance was read at 765 nm, all the analysis was performed thrice. The total phenolic content was estimated using the linear regression equation from the standard calibration curve further mean \pm SD ($n = 3$) was calculated and expressed as mg/g gallic acid equivalent (GAE).

Estimation of saponins

The saponin content of the extracts was measured as described by Le et al. [13]. Quillaia was used to obtain the standard calibration curve. The sample (1 mg/ml) was mixed with 500 μ L of 8% vanillin and 500 μ L of 72% sulfuric acid and incubated at 60 °C for 10 min after cooling to room temperature absorbance was recorded at 544 nm. The total saponin content was quantified using the linear regression equation obtained from the standard graph of quillaia further mean \pm SD ($n = 3$) was quantified as mg/g quillaia equivalent (QE). All determination was carried out in triplicates.

2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay

The ability of *A. chinense* bulb and leaf extracts to scavenge free radicals was assessed according to the standard DPPH method [14]. Ascorbic acid was used as the standard and the assay was performed in triplicate. One milliliter of the varying concentrations (1–100 μ g/ml) was added to 3.0 ml of freshly prepared DPPH (0.06 mM). The mixture was further incubated in the dark for 15 min at room temperature (27 ± 2 °C). The decrease in the absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is absorbance of control (DPPH) and A_1 absorbance of extracts

Table 1 Quantitative analysis of *A. chinense* bulb and leaf extracts

Sl. No.	Quantitative analysis (mg/g)	Linear regression equation for standard	Leaf	Bulb
1	Alkaloids	$Y = 0.0003X + 0.0009$, $R^2 = 0.9996$ (atropine)	77.000 \pm 0.333	77.830 \pm 0.289
2	Flavonoids	$Y = 0.0029X + 0.0031$, $R^2 = 0.9992$ (quercetin)	6.862 \pm 0.597	9.275 \pm 0.398
3	Phenol	$Y = 0.0127X + 0.0252$, $R^2 = 0.9991$ (gallic acid)	18.409 \pm 0.273	20.100 \pm 0.069
4	Saponins	$Y = 0.0002x + 0.003$, $R^2 = 0.999$ (quillaia)	375.000 \pm 0.577	163.750 \pm 0.433

Values are mean \pm SE ($n = 3$)

The calibration curve for inhibition was prepared and IC_{50} values were calculated.

Antimicrobial analysis by disk diffusion method

The antimicrobial activity of the bulb and leaf extract was screened by the agar disk diffusion method. Muller-Hinton agar (MHA) (Hi-Media) plates were swabbed with gram-positive *Staphylococcus aureus* (MTCC916) and gram-negative *Pseudomonas aeruginosa* (MTCC741) bacteria and fungi *Aspergillus niger* (MTCC281) in Sabouraud dextrose agar (SDA) plate [15]. Plant extracts of 1 mg/ml concentration were prepared in 0.1% dimethyl sulfoxide (DMSO). Four wells measuring 6 mm were bored in the inoculated media with a sterile cork-borer. The disks were loaded with 30 μ L of the extract and placed on the MHA and SDA plate. The Petri-plates were further incubated at 37 °C for 24 h for bacteria and 22 °C for 48 h for fungi. At the end of incubation, zones of inhibition (ZOI) were observed around the disk and measured with a transparent ruler in millimeters. Streptomycin (30 μ g/ml) and fluconazole (1 mg/ml) were used as the positive control for bacteria and fungi respectively. 0.1% DMSO was used as a negative control.

GC-MS analysis

To obtain the complete chemical profile, analysis was performed using GC-MS (SHIMADZU QP2010S) system, with the Rxi-5Sil MS column; length 30 meters, and helium (99.99%) as a carrier gas at a flow of 1.00

Table 2 Antioxidant activity measured by DPPH scavenging

Concentration (μ g/ml)	Ascorbic acid	Leaf extract	Bulb extract
1	9.630 \pm 0.057	1.238 \pm 0.133	1.972 \pm 0.010
10	15.313 \pm 0.067	3.665 \pm 0.007	2.369 \pm 0.056
25	29.390 \pm 0.115	5.077 \pm 0.015	4.317 \pm 0.012
50	49.127 \pm 0.037	7.502 \pm 0.018	6.594 \pm 0.032
75	62.160 \pm 0.034	9.036 \pm 0.139	7.479 \pm 0.010
100	83.167 \pm 0.072	10.729 \pm 0.260	8.731 \pm 0.006
IC_{50}	55.118	533.337	678.347

Values are mean % inhibition \pm SE ($n = 3$; $p < 0.05$)

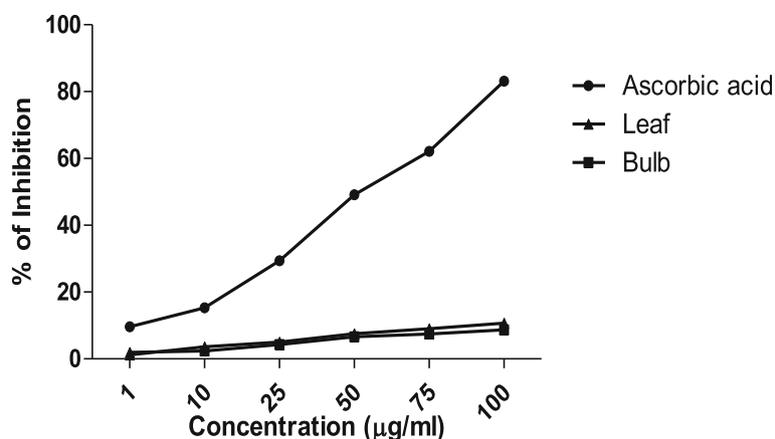


Fig. 2 Scavenging activity of ascorbic acid, bulb, and leaf extract of *A. chinense*

mL/min. Injection mode was splitless and sampling time 2.00 min; flow control mode was linear velocity at a split ratio 100.0 MS and ID 0.25 mm. The ion source temperature was set at 200.00 °C, interface temperature 280.00 °C, and oven temperature was programmed at 70.0 °C. Solvent cut time was 6.50 min, start time 7.00 min, end time 35.75 min, event time 0.50 s, and scan range 50–500 m/z.

Identification of compounds

The identification of chemicals was based on the peaks of the compounds at different mass-to-charge ratios. Results were obtained in accordance with the mass spectral library of National Institute of Standards and Technology, NIST-11. The unknown spectrum was compared with the standard spectrum existing in the database of the WILEY 8 library.

Statistical analysis

All the estimation was carried out in triplicates. The data obtained in this study were analyzed using a one-way analysis of variance (ANOVA) by using Prism V. 5.00 (Graphpad Inc. USA). The results were expressed as mean ± SE. Significance value (P) < 0.05 was selected as a point of minimum significance rate.

Results

Quantitative analysis

Phytochemicals have been analyzed quantitatively. The total alkaloid, flavonoid, phenol, and saponin concentration of the bulb and leaf extract are presented in Table 1.

DPPH assay

The activity of *A. chinense* extract against free radicals is shown in Table 2 and Fig. 2. The *A. chinense* extract showed less scavenging capacity both in the leaf ($IC_{50} = 533.337 \mu\text{g/ml}$) and bulb ($IC_{50} = 678.347 \mu\text{g/ml}$) when compared to standard ascorbic acid ($IC_{50} = 55.118 \mu\text{g/ml}$) with significant difference ($p < 0.05$).

Antimicrobial activity

Antimicrobial activity was observed in all the species tested (Table 3, Fig. 3). The extracts of *A. chinense* exhibited exquisite antibacterial activity with 15 mm (leaf) and 10 mm (bulb) ZOI in gram-positive (*Staphylococcus aureus*) and 20 mm (leaf) and 16 mm (bulb) ZOI in gram-negative bacteria (*Pseudomonas aeruginosa*). The absence of ZOI was interpreted as the absence of activity. In *Aspergillus niger*, bulb extract showed 7 mm ZOI and 14 mm ZOI in the leaf extract.

Table 3 Diameter of zones of inhibition (mm) of bulb and leaf extract of *A. chinense* against tested microorganisms

Sample/test organism	<i>Staphylococcus aureus</i> (gram-positive) (mm)	<i>Pseudomonas aeruginosa</i> (gram-negative) (mm)	<i>Aspergillus niger</i> (mm)
Leaf	15	20	14
Bulb	10	16	7
Positive control	16	18	16
Negative control	NA	NA	NA

NA no activity



Fig. 3 Growth inhibition of *S. aureus*, *P. aeruginosa*, and *A. niger* caused by *A. chinense* bulb (Hex-1) and leaf (Hex-2) extracts

GC-MS analysis

GC-MS analysis is a superior technique to identify the phytoconstituents. Nine compounds were identified from hexane leaf extract and twenty-eight compounds from hexane bulb representing 100% of the total extract. The identification was based on retention time, peak area, molecular formula, molecular weight, and molecular structure which are shown in (Figs. 4 and 5, Tables 4 and 5). The GC-MS analysis of hexane leaf extract revealed the presence of this compounds viz., perhydrofarnesyl acetone, phytol, nonadecane, 2-methyl-, heptadecyl heptafluorobutyrate, eicosane, 7-hexyl-, octadecyl trifluoroacetate, gamma-tocopherol, tetratetracontane, heptadecane, 2,6-dimethyl- and the compounds identified in the bulb are 9, 12 octadecadienoic acid, methyl ester, tritetracontane, 1-ethylsulfanylmethyl-2,8,9-trioxo-5-aza-1-sila-bicyclo[3.3.3]undecane, 3,6,9,12-tetraoxahexadecan-1-ol, 9,12-octadecadienoic acid, ethyl ester, 3,4,4a,5,6,7,8a-octahydro-spiro[cyclohexane-1,4'-(2H-1,3-benzothiazine)]-2-thione-8a-ol, heptadecane, 4,8,12,16-tetramethylheptadecan-4-olide, hexadecanoic acid, 2-methylpropyl ester, nonadecane, 2-methyl-, hexadecanoic acid,3-[(trimethylsilyl)oxy] propyl ester, hexadecanoic acid-2-hydroxyl-1- (hydroxy methyl) ethyl ester, eicosane, 10-methyl-, diisooctyl phthalate, n-propyl 9,12-octadecadienoate, eicosane, 2-methyl-,

cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl-, 1- heneicosanol, 1-heptacosanol, 2-methyloctacosane, octadecyl trifluoroacetate, eicosane, 7-hexyl-, n-tetracosanol-1, tetra-tetracontane, docosane, 11-butyl-, heneicosane, n-nonadecanol-1, and vitamin E.

Discussion

In the quantitative estimation of *A. chinense* bulb and leaf, high content of saponin was observed. Saponin possesses antibiotic, insecticidal, and fungicidal activity [16]. Steroidal saponins isolated from *A. chinense* bulb possess anti-tumor property [7]. Saponins are cytotoxic and inhibit the migration ability of B16 and 4T1 cells [4]. Laxogenin a saponin isolated from *A. chinense* bulb have anti-tumor property in stage-two lung carcinogenesis [8]. Therefore, *A. chinense* can be used as a potential plant for the extraction of saponin for medicinal and pharmaceutical use.

Alkaloids have been reported to have anticancer, antibacterial, antiviral, and antifungal activity [17]. Acridinium bromide a drug from alkaloid is used to treat chronic obstructive pulmonary disease; atropine and scopolamine are alkaloid derivatives used in traditional medicine for treating asthma [18]. *A. chinense* showed a moderate content of alkaloids which

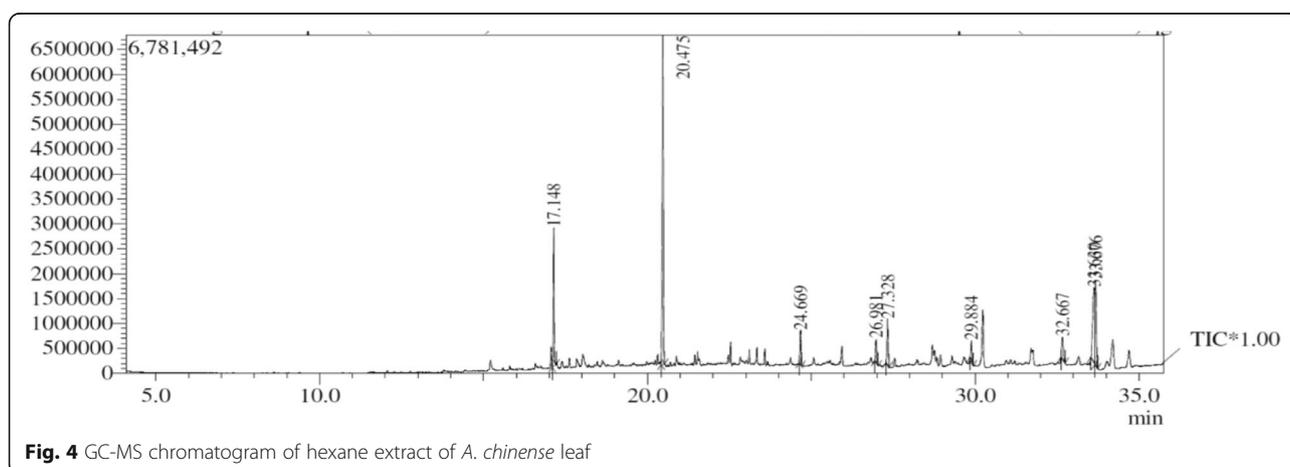
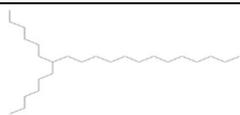
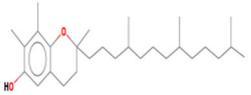


Fig. 4 GC-MS chromatogram of hexane extract of *A. chinense* leaf

Table 4 Phytocompounds identified in the hexane extract of *A. chinense* leaf by GC-MS analysis

Retent ion time	Name of the compound	Peak Area %	Mol. Wt.	Structure	Nature of compounds
17.148	Perhydrofarnesyl acetone C ₁₈ H ₃₆ O	14.76	268		Sesquiterpenoids
20.475	Phytol C ₂₂ H ₄₂ O ₂	35.76	338		Diterpene
24.669	Nonadecane, 2-methyl- C ₂₀ H ₄₂	3.61	282		Alkane
26.981	Heptadecyl heptafluorobutyrate C ₂₁ H ₃₅ F ₇ O ₂	3.03	452		Alkyl
27.328	Eicosane, 7-hexyl- C ₂₆ H ₅₄	6.56	366		Alkane
29.884	Octadecyl trifluoroacetate C ₂₀ H ₃₇ F ₃ O ₂	2.73	366		Esters
32.667	.gamma.-Tocopherol C ₂₈ H ₄₈ O ₂	4.27	416		Vitamin E
33.630	Tetratetracontane C ₄₄ H ₉₀	18.49	619		Alkane
33.676	Heptadecane, 2,6-dimethyl- C ₁₉ H ₄₀	10.79	268		Alkane

property [28]. Eicosane, 10-methyl with a peak area of 12.06% appeared at 24.850 min, an alkane which has high antioxidant [31]. Heneicosane with a peak area of 8.46% reported at 33.939 min is an aliphatic hydrocarbon; it belongs to the higher alkane group having biopesticidal property [32].

The GC-MS revealed that the bulb and leaves have a different bio-constituent; only three compounds tetratetracontane, nonadecane, and 2-methyl-octadecyl trifluoroacetate appeared in both the extract. Our findings on the chemical composition of hexane bulb and leaf extract did not correlate with the previous reports on the essential oil [5, 6] and no sulfide-

containing compounds were identified in the crude extract.

Conclusion

The quantitative analysis showed a high content of saponins in the leaf extract. GC-MS analysis of the extract suggests that numerous medicinally important bioactive constituents are present in *A. chinense* leaf and bulb, with high terpene content in the leaf and alkanes in the bulb.

These important findings revealed through our study suggest isolation of bioactive compounds, and screening its activity will have tremendous

Table 5 Phytochemicals identified in the hexane extract of *A. chinense* bulb by GC-MS analysis

Retention Time	Peak Area %	Name of the compound	Mol. weight	Mol. structure	Nature of compound
20.344	0.29	9,12-Octadecadienoic acid, methyl ester C ₁₉ H ₃₄ O ₂	294		Linoleic acid ester
20.421	0.34	Tritetracontane C ₄₃ H ₈₈	605		Alkane
20.817	0.41	1-Ethylsulfanylmethyl-2,8,9-trioxo-5-aza-1-sila-bicyclo[3.3.3]undecane C ₈ H ₁₉ N ₀ SSi	249		Aromatic compound
20.985	0.66	3,6,9,12-Tetraoxahexadecan-1-ol C ₁₂ H ₂₆ O ₅	250		Terpene alcohol
21.113	0.56	9,12-Octadecadienoic acid, ethyl ester C ₂₀ H ₃₆ O ₂	308		Polyenoic fatty acid
21.184	0.36	3,4,4a,5,6,7,8,8a-Octahydro-spiro[cyclohexane-1,4'-(2H)-1,3-benzothiazine]-2-thione-8a-ol C ₁₃ H ₂₁ NOS ₂	271		Aromatic compound
22.680	4.97	Heptadecane C ₁₇ H ₃₆	240		Alkane
23.223	0.38	4,8,12,16-Tetramethylheptadecan-4-olide C ₂₁ H ₄₀ O ₂	324		Terpenoids
23.490	0.45	Hexadecanoic acid, 2-methylpropyl ester C ₂₀ H ₄₀ O ₂	312		Fatty acid
23.708	1.86	Nonadecane, 2-methyl- C ₂₀ H ₄₂	282		Volatile heterocyclic hydrocarbon
24.507	3.46	Hexadecanoic acid, 3-[[trimethylsilyloxy]propyl ester C ₂₂ H ₄₆ O ₃ Si	386		Fatty acid ester
24.850	12.06	Eicosane, 10-methyl- C ₂₁ H ₄₄	296		Alkane
25.050	1.08	Hexadecanoic acid-2-hydroxy-1-(hydroxymethyl) ethyl ester C ₁₉ H ₃₈ O ₄	330		Amino compound
25.219	0.63	Diisooctyl phthalate C ₈ H ₁₄ (C ₈ H ₁₇ COO) ₂	390		Plasticizer compound
25.421	0.81	n-propyl 9,12-octadecadienoate C ₂₁ H ₃₈ O ₂	322		Fatty acid ester
26.112	2.95	Eicosane, 2-methyl- C ₂₁ H ₄₄	296		Alkane
26.753	0.80	Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl- C ₄ H ₇ Cl ₂	167		Carboxylic acid
27.176	5.76	1-Heneicosanol C ₂₁ H ₄₄ O	312		Primary fatty alcohol
27.280	2.58	1-Heptacosanol C ₂₇ H ₅₆ O	396		Primary fatty alcohol
27.601	21.30	2-methyloctacosane C ₂₉ H ₆₀	408		Alkane
28.964	3.22	Eicosane, 7-hexyl C ₂₆ H ₅₄	366		Aliphatic hydro compounds
30.104	5.57	Octadecyl trifluoroacetate C ₂₀ H ₃₇ F ₃ O ₂	366		Esters
30.204	1.65	n-tetracosanol-1 C ₂₄ H ₅₀ O	354		Primary fatty alcohol
30.497	14.05	Tetratetracontane C ₄₄ H ₉₀	619		Alkane
31.999	2.40	Docosane, 11-butyl- C ₂₆ H ₅₄	366		Alkane
33.430	0.91	Octadecyl trifluoroacetate C ₂₀ H ₃₇ F ₃ O ₂	366		Esters
33.939	8.46	Heneicosane C ₂₁ H ₄₄	296		Aliphatic hydrocarbon
34.022	0.93	n-Nonadecanol-1 C ₁₉ H ₄₀ O	284		Alcoholic compound
34.449	1.10	Vitamin E C ₂₉ H ₅₀ O ₂	430		Vitamin

benefits, considering its availability, that it is edible and has medicinal value, and that it is non-toxic; however, a toxicological analysis would be of necessity to develop safe drugs. This work represents an initial step to understand the plant phytochemical constitution which could facilitate further investigation.

Abbreviations

AlCl₃: Aluminum trichloride; AE: Atropine equivalent; DMSO: Dimethyl sulfoxide; DPPH: 2,2-Diphenyl-1-picrylhydrazyl; FC: Folin-ciocalteu; GAE: Gallic acid equivalent; GC-MS: Gas chromatography-mass spectroscopy; HCl: Hydrochloric acid; MHA: Mueller-Hinton agar; NA: No activity; NaOH: Sodium hydroxide; NaNO₂: Sodium Nitrite; NIST: National Institute of Standards and Technology; QE: Quercetin equivalent; QE: Quillaia equivalent; SDA: Sabouraud dextrose agar; SE: Standard error; UV-Vis: Ultraviolet-visible; ZOI: Zone of inhibition

Acknowledgements

The authors are thankful to the Department of Botany, Bangalore University, Bangalore 560056, for providing facilities, and Central Instrumentation Unit, Kerala Forest Research Institute (KFRI), Peechi, Thrissur, Kerala, India, for GC-MS analysis.

Plant authentication

The plant was authenticated by Dr. V Rama Rao, Regional Ayurveda Research Institute for Metabolic Disorders, Central Council for Research in Ayurvedic Sciences, Ministry of AYUSH, Govt. of India, Bengaluru 560109. The authenticated sample was preserved with the specimen voucher number RRCBI-mus 244.

Authors' contributions

TR performed all the above analysis and drafted the manuscript. SR participated in performing the analysis and interpretation of the data. RMS participated in performing the analysis. VS participated in the design, drafting, and critically revising the manuscript. All authors have read and approved the manuscript.

Funding

The first author is thankful to the University Grant Commission (UGC) Maulana Azad National Fellowship (MANF) of the Government of India for providing Senior Research Fellowship (SRF).

Availability of data and materials

The data used to analyze the findings of this study are available from the corresponding author upon request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 9 March 2020 Accepted: 31 August 2020

Published online: 27 November 2020

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